



## SURFACE COLONIZATION OF *CANDIDA* SPP. IN CONVENTIONAL AND DOUBLE-LAYER COMPLETE DENTURES – A PILOT STUDY

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### ABSTRACT:

**Background:** The scientific interest in dominant *Candida* species colonizing the surface of conventional and double-layer dentures (D-LCDs) is still topical. Numerous studies are comparing fungal colonization in both types of removable dentures.

**Aim:** Performing a comparative analysis of the colonization of *Candida* spp. on the dentures surfaces of the conventional (CCDs) and the double-layer dentures (soft-lined) complete dentures (D-LCDs) over six months, placing them under the same intraoral *in vivo* conditions.

**Methods:** For isolating *Candida* spp., specimens were obtained from inner denture surfaces using two sterile swabs, one for the upper complete denture (n=28) and one for the lower complete denture (n=28). The culture-based *Candida* detection method with CHROM agar was used to identify *Candida* species.

**Results:** In the third month after dentures delivery *C. Albicans* is the predominant *Candida* spp. found on both types of complete dentures surface and isolated in 46 % of the patients with conventional complete dentures and 43 % with double-layer dentures (p>0.05). In the sixth month of the finished prosthetic treatment, combinations of *C. Albicans* and non-albicans *Candida*, predominantly *C. Tropicalis*, *C. Glabrata* and *C. Parapsilosis*, were found in 43 % of D-LCDs and 45 % of CCDs (p>0.05).

**Conclusion:** There was no difference in colonization based on the different materials used for denture fabrication. However, there is a significant proportional change for all studied *Candida* spp. over the period (3-6 months after delivery), irrespective of the denture material used.

**Keywords:** complete dentures, soft denture liner, resilient liner, *Candida* spp.,

### INTRODUCTION

The ability of the resilient lining materials to control the stress-strain distribution on the oral mucosa makes them suitable for a wide range of applications in prosthetic dentistry. Completely edentulous patients with excessive bone resorption, thin, non-resilient mucosa and exostoses are indicated for a specific clinical approach, namely, the fabrication of double-layer complete dentures (D-LCDs) (hard acrylic resin complete dentures, lined with resilient material). This type of denture distributes the masticatory pressure uniformly, reducing mucosal injury. The great marginal seal improves the dentures' retention and stability by engaging additional retentive areas. [1] The high humidity and temperature in the oral cavity, the hygienic regimen, and the composition of saliva are conditions for bacterial and fungal colonization (mainly *Candida* spp.) on the surface of removable dentures. Several authors have reported that colonization in double-layer dentures (D-LDs) dentures lined with resilient materials is higher due to the surface characteristics (roughness, hydrophilicity/hydrophobicity), type of polymerization and chemical composition of the soft materials. [1-7]. Chladek G, et al. [2] reported that Molloplast B inhibited fungal growth, and Valentini F, et al. [8] found no significant differences in the biofilm composition between the two types of dentures in healthy patients.

Generally, fungal species are divided into *Candida Albicans* and non-albicans *Candida*, including but not limited to *C. Glabrata*, *C. Tropicalis*, and *C. Parapsilosis*. *C. Albicans* is the most common opportunistic species in persons [1, 7, 9]. Olson ML, et al. [10] and Fernández-Pereira J, et al. [11] observed strong adhesion of *C. Albicans* and *C. Glabrata* to prosthetic surfaces as a result of their pronounced hydrophobicity and the release of adhesives by *C. Glabrata*. A previous study published in 2017 found an increased presence of *C. tropicalis* in the saliva of patients with double-layer complete dentures [13]. The presence of *Candida* spp. in the oral cavity or on the surfaces of the dentures is not a problem in itself. Still, it can cause the development of denture stomatitis. Still, other predisposing factors must be considered in the development of this

disease, such as night use of dentures, xerostomia, smoking, poor glycemic control, and poor denture hygiene [9, 15-17].

According to M. Patel [17], the regular use of antimicrobial agents for denture hygiene maintains the low number of *Candida* spp. and reduces their virulence. According to the author, the level of saliva flow and antimicrobial peptides (sIgA and LF) in the saliva, the indisputable fact that an anaerobic and acidic environment is created under the dentures, as well as the porosity and hydrophilicity/hydrophobicity of the materials for the dentures making, also play a major role in the development of infection. Nearly 65% of denture wearers have denture stomatitis, which may be asymptomatic [18]. In a study by Taebunpakul & Jirawechwongsakul [19], *Candida* spp. was found in 81.7% of denture wearers. Nearly 55% of patients had symptoms of denture stomatitis. According to the authors, the amount of fungi is not a determinant of the degree of this disease, according to Newton's classification. According to some authors, the main causative agent of denture stomatitis is *C. Albicans*, followed by *C. Glabrata* and *C. Tropicalis* [13, 14, 17, 19, 20], with *C. Tropicalis*, observed in the more severe course of the disease [10]. An extreme form of denture stomatitis can be a risk for serious systemic infections [18].

**The study aimed** to perform a comparative analysis of the colonization of *Candida* spp. on the denture surfaces of the conventional (CCDs) and the double-layer dentures (soft-lined) complete dentures (D-LCDs) over six months, placing them under the same intraoral *in vivo* conditions.

The study (null) hypothesis was: There would be no difference in the colonization of *Candida* spp. between groups, considering the materials used to make the denture bases and there would be no change in the proportion of *Candida* spp. colonies for the different periods.

## MATERIAL AND METHODS

### 1. Patient selection

In the Faculty of Dental Medicine, Medical University - Sofia, 28 patients, completely edentulous (7 male and 21 female), aged between 48 and 90 years, were treated with complete dentures.

Patients with systemic diseases (asthma, type I and uncontrolled type II diabetes, Sjögren's syndrome, immunodeficiency conditions), patients who used antibiotics in the previous three months, and patients under radiotherapy or chemotherapy in the previous six months were not included in the study. Patients diagnosed with denture-related

stomatitis due to wearing old removable dentures, smokers, and systematically drinking alcohol were also not included in the study.

### 2. Clinical and microbiological methods

Each patient was provided a full set of dentures, including a conventional upper complete denture and a soft-lined lower complete denture. The patients were assigned into two groups, depending on the soft denture liner used for the lower dentures.

**The first group (group A)** (n=15) included patients treated with the conventional complete denture for the upper jaw [Meliodent acrylic resin (Kulzer)] and full denture, lined with a heat-cured silicone-based soft material [Molloplast B (Detax, Germany)] for the lower jaw.

**The second group (group B)** (n=13) included patients treated with the conventional complete denture for the upper jaw [Meliodent acrylic resin (Kulzer)] and complete denture, lined with a self-curing silicone-based soft material [Īegabase (Dreve, Germany)] for the lower jaw.

Before inclusion in the study, all patients signed an informed consent form. The Research Ethics Commission approved the study "KENIMUS" (Statement No. 21/2016).

The study participants were given a supply of the same denture cleaning tablets (Protefix, Germany) for six months and verbal and written usage instructions.

For isolating *Candida* spp., specimens were obtained from inner denture surfaces using two sterile swabs, one for the upper complete denture (n=28) and one for the lower complete denture (n=28). The culture-based *Candida* detection method with CHROM agar was used to identify *Candida* species.

### 3. Statistical Analysis

Descriptive statistics and graphical analyses were used to characterize the data. A Welch t-test was used to assess age distribution across different relining materials. A chi-square goodness of fit test was used to assess the proportion distributions, and a chi-square test independence was used to evaluate the association between different variables. A McNemar test was utilized to evaluate isolated species at different time points. The alpha level was set at 95% for all statistical tests. Where necessary, a grouping of the resulting variables was performed. The software used for all statistical procedures and graphics generation was R Core Team (2018) 4.05 (Lucent Technologies, Auckland, New Zealand Lucent Technologies, Auckland, New Zealand).

## RESULTS

The Age, Gender and Material characteristics of the sample are presented in Table 1.

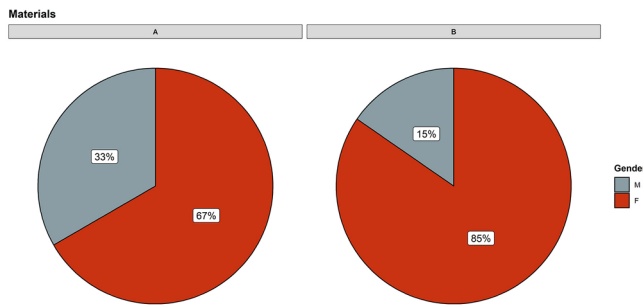
**Table 1.** Age, Gender and Material characteristics of the sample.

| Patient count | Gender |    |        |    | Age   |       | Material |    |       |    |
|---------------|--------|----|--------|----|-------|-------|----------|----|-------|----|
|               | Male   |    | Female |    | Mean  | SD    | A        |    | B     |    |
|               | count  | %  | count  | %  |       |       | count    | %  | count | %  |
| N=28          | 7      | 25 | 21     | 75 | 68.64 | 10.86 | 15       | 54 | 13    | 46 |

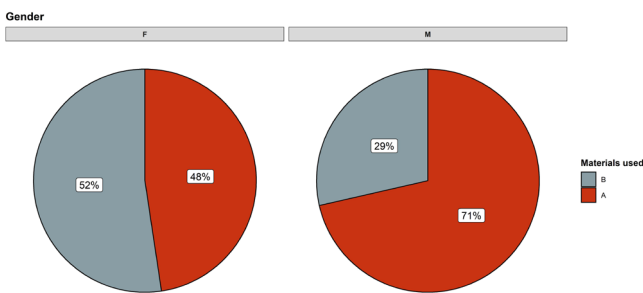
A chi-square test showed a statistically significant difference in the Distribution of gender within the studied sample [ $\chi^2(1) = 7, p = 0.008$ ]. The female (n=21) participants are three times more than their male counterparts (n=7).

Figure 1 a, b depicts the Distribution of different sexes within the materials and the materials within the gender variable.

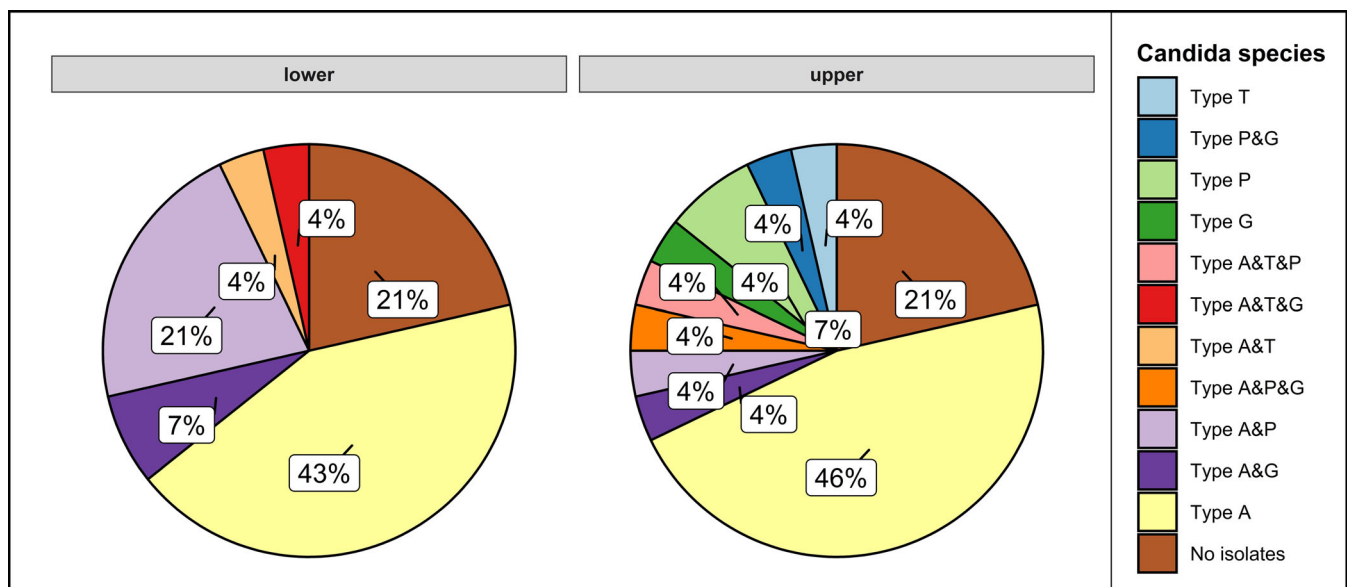
**Fig. 1a.** Distribution of different sexes within the materials.



**Fig. 1b.** Distribution of different sexes within the gender.



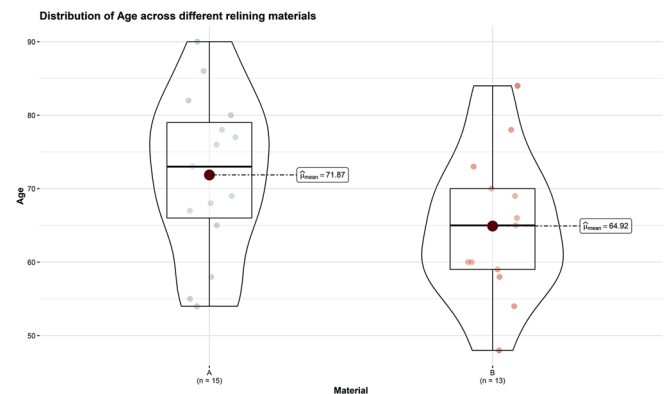
**Fig. 3a.** Distribution of *Candida* spp. in both types of dentures in the third month.



The mean age of the study sample was  $68.64 \pm 10.86$  years. A two-sample Welch t-test was used to compare the mean age between the two tested materials. No significant difference was observed [ $t_{\text{Welch}}(25.928) = 1.763, p = 0.09$ ].

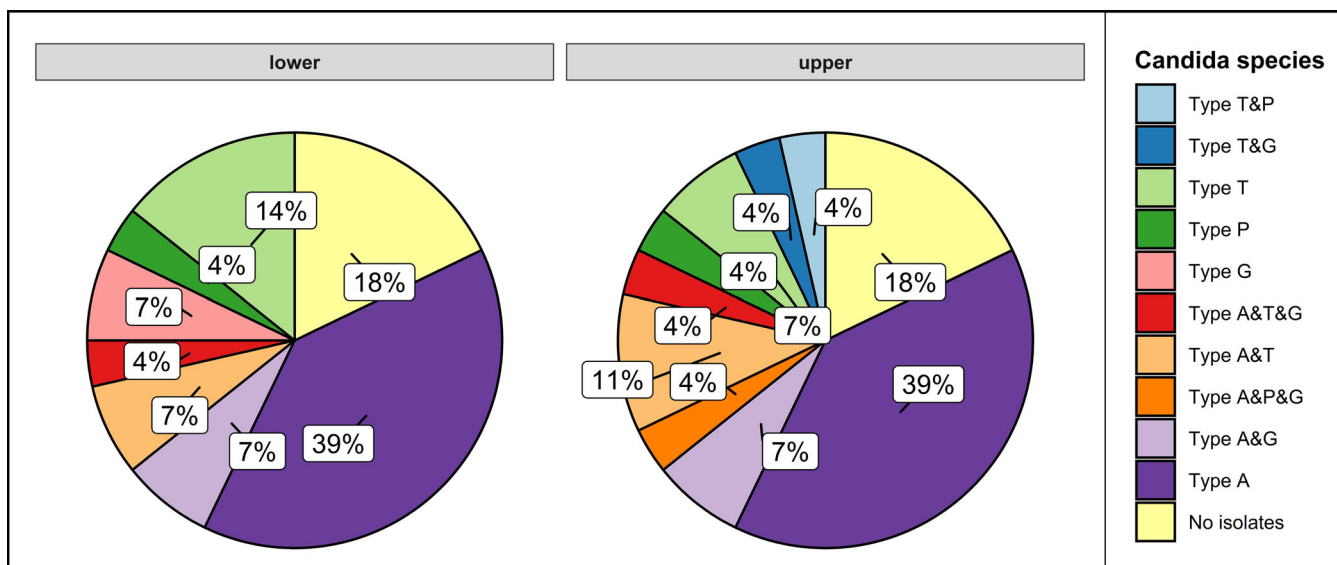
The Distribution of different isolated microorganisms across the variables “Denture” and “Periods of isolation and culturing” are presented in Figure 2.

**Fig. 2.** Distribution of different isolated microorganisms.



The results from the non-stimulated saliva collected before the delivery of the dentures revealed no *Candida* spp. for both study groups.

**Fig. 3b.** Distribution of *Candida* spp. in both types of dentures in the sixth month.



Due to the small number of different isolated combinations of *Candida* spp., grouping was performed to enable the use of association tests. No significant difference was detected between the different isolated *Candida* spp. based on the materials used in the upper and lower dentures and between the two soft relining materials ( $p > 0.05$ ), irrespective of whether individual *Candida* spp. or grouping was used for the statistical tests (Fig. 3 a, b). A McNemar test assessed the *Candida* spp.—colonization change between the third and sixth months. A significant change was observed when evaluating all isolated *Candida* subspecies – Table 2.

**Table 2.** Change in *Candida* spp. colonization between the third and sixth month.

| <i>Candida</i> spp. | $\chi^2$ | P value |
|---------------------|----------|---------|
| Albicans            | 4.2      | < 0.05  |
| Glabrata            | 27.2     | < 0.001 |
| Tropicans           | 28.8     | < 0.001 |
| Parapsilosis        | 23.8     | < 0.001 |

The number of isolated *Albicans* species decreased significantly in the sixth month, whereas it increased for all other subspecies.

## DISCUSSION

The ability of resilient lining materials to control the stress-strain Distribution over the oral mucosa and to provide a cushioning effect to the cyclic masticatory forces make them suitable for a wide range of applications in prosthetic dentistry. One of the main disadvantages of these materials is microbial colonization. Resilient materials can be acrylic or silicone-based, heat- or self-curing [1, 2]. In the scientific literature, the increased roughness of these materials and the hydrophilicity of acrylic-based resilient materials are discussed as the main reason for the increased

microbial colonization [1-6, 8]. On the other hand, silicone-based soft materials are hydrophobic and are recommended for longer-term use, although they lag behind elastic properties compared to acrylic-based elastic materials [3-6].

Considering the characteristics of the materials used to fabricate conventional and double-layer complete dentures, we decided to place these two types of dentures under the same *in vivo* conditions. Thus, verifying the proposition of several authors about the increased microbial colonization in the double-layer complete dentures, using two types of resilient materials, both silicone-based.

The different *Candida* species were identified in CHROMagar *Candida* (ELTA, Bulgaria) solid culture medium, a method used by some authors [8, 9, 14, 15, 20]. The medium contains chromogenic substrates that react with enzymes produced by various pathogens and produce colonies of different colours and morphology. CHROMagar *Candida* successfully identified 95% of *C. Albicans*, 94% of *C. Glabrata*, 100% of *C. Tropicalis* and 100% of *C. Krusei* isolated from other species based on their colour and morphology.

*Candida* spp. is part of the normal human microbiota. It is often found on the mucosa of the oral cavity and gastrointestinal tract but can become pathogenic if the host's microbiome or immune system is compromised. *C. Albicans* is a fungus most often found on mucosal and prosthetic surfaces and is considered the main cause of denture stomatitis [7, 8, 10, 11, 15, 17, 18]. *C. Tropicalis* is frequently isolated in patients with poor glycemic control [9]. *C. Albicans* is considered hydrophilic and expected to colonize hydrophilic surfaces, such as resilient acrylic-based lining materials. In contrast, *C. Glabrata* is hydrophobic and colonized more on hydrophobic surfaces such as an acrylic resin (PMMA) for manufacturing the base of the dentures and the silicone-based resilient materials, and mainly the heat-curing ones, in contrast to the self-curing ones, which are considered more hydrophilic [6]. As another main reason for the increased colonization in D-LCDs, some authors define the increased roughness of the resilient materials [5]. Gad et al. [6] found

a roughness for the acrylic resin (PMMA) of about 2.07  $\mu\text{m}$  and for the soft lining materials of 3.84  $\mu\text{m}$ , regardless of whether they were heat- or self-cured.

In the present study, the *Candida* specimens were grouped into *C. Albicans* and non-albicans *Candida*. In the third month, after dentures delivery, *C. Albicans* was isolated and identified in more than 46 % of conventional dentures and 43 % of soft-lined ones. These data obtained by us reject the statement of most authors [1 – 7] about increased microbial colonization in double-layer complete dentures but are following the fact that the *C. Albicans* is a major inhabitant of the oral cavity [5 - 7, 13, 14, 17]. The obtained results confirm the null hypothesis and are in contradiction with the conclusion of Gad et al. [6] and Tasopoulos et al. [5] that double-layer complete dentures (D-LCDs) retain a greater amount of fungus compared to conventional ones (CCDs).

Over time in D-LCDs, we observed a trend for a displacement of *C. Albicans* by other *Candida* spp., such as *C. Tropicalis*, *C. Glabrata* and *C. Parapsilosis*, which is in accordance with the results of Valentini et al. [8]. Combinations of *C. Albicans* and non-albicans *Candida*, predominantly *C. Tropicalis*, *C. Glabrata* and *C. Parapsilosis*, were found in 36% of D-LCDs in the third month and 34% of CCDs after denture delivery. That combination was found in 45 % of CCDs and 43 % of D-LCDs in the sixth month. This result is in accordance with Rodríguez-Archilla et al. [9], who found such combinations on the denture surfaces of nearly 43% of healthy patients without systemic diseases and 66.7% of patients with poor glycemic control.

*C. Albicans*, combined with *C. Glabrata*, is often isolated at the infection places and complicates the inflammatory process due to possible synergism [10]. Such a combination Ozaki et al. [15] find in 60% of fungal colonies. The present study found such a combination in the third month after prosthetic treatment in 7.1% of D-LCDs and 3.6% of CCDs, and 7% of both complete denture types in the sixth month. These combined biofilms are more resistant to treatment due to the presence of non-albicans *Candida* that are resistant to first-line antifungal agents [8, 20]. According to Fernández-Pereira et al. [11] and Shantal et al. [12], *C. Glabrata* expresses many adhesives-encoding genes that help improve adhesion to various surfaces. In recent years, this fungus has received increasing attention, as it is considered one of the possible causes of head and neck cancer [15].

The present research observed a trend for the considerable quantitative presence of *Candida* spp in the third and sixth months after denture delivery in both types of complete dentures. In the third month after denture delivery in the patients from group A, dentures lined with Molloplast B (heat-polymerized silicone-based resilient material, (Detax, Germany)), we observed a trend for least (40%) amount of *Candida* spp. The amount of *Candida* spp. in Megabase (room-polymerized silicone-based resilient material, (Dreve, Germany)) – lined dentures in group B patients was comparable to that of the conventional com-

plete dentures at both the third and sixth months after prosthetic treatment (61.5 % [Megabase, (Dreve, Germany)] / 53, 6 % [CCD] - third month ( $p>0.05$ ) and 64.3 % [CCD]; 66.7 % [Molloplast B, (Detax, Germany)] and 69.2 % [Megabase, (Dreve, Germany)] - sixth month ( $p>0.05$ )). This finding is in accordance with the results of Gad et al. [6], who reported less retention of *C. Albicans* to heat-cured silicone-based resilient material but attributed this to the higher hydrophilicity of the room-polymerized silicone-based resilient material. The lower detected amount of *Candida* spp. in Molloplast B (Detax, Germany) in the third month after prosthetic treatment finds an explanation in a publication by Chladek et al. [2], which describes the findings of other authors on the release of dibenzoyl peroxide from Molloplast B into the environment. It is possible that this reaction is only at the beginning and decreases with time, which would explain the results we obtained. In a previous study conducted in 2017, we found that *C. Tropicalis* in the saliva of patients with soft-lined complete dentures increased over time. The quoted study found that the development of denture stomatitis in patients with D-LCDs is mainly due to *C. Albicans* and *C. Tropicalis* [13].

**Instructions and advice to the patients:** Patients prone to biofilm build-up on their dentures due to the type of denture material and/or their general health are advised to enhance denture and oral hygiene. Patients are advised not to wear their dentures at night, as the cleaning functions of saliva are missing, and to take probiotics to stabilize the microbiome. Patients should be instructed to clean their dentures thoroughly after eating and to store them in a tablet solution overnight.

Limitations of the present study include the small number of patients studied, especially in group B, and the lack of objective data on glycemic control. Type 2 diabetes is not always known to patients, especially older ones. Furthermore, the study's design does not consider a possible carry-over effect when comparing the heat-polymerized PMMA upper dentures and the relined lower dentures. This might explain the similar colonization results obtained for both groups.

## CONCLUSION

Considering the limitations of the present study and the results obtained, there was no difference in colonization based on the different materials used for denture fabrication. However, there is a significant proportional change for all studied *Candida* spp. over the studied period (3-6 months after delivery), irrespective of the denture material used.

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